Biocidin® LSF
Potent Broad-Spectrum Liposomal Formula
Scientific Validation of Ingredients

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Active Ingredients:

Biocidin® Proprietary Blend

Bilberry fruit extract (*Vaccinium myrtillus*), Grape seed extract (*Vitis vinifera*), Shiitake mushroom extract (*Lentinula edodes*), Goldenseal root (*Hydrastis canadensis*), Noni fruit extract (*Morinda citrifolia*), Garlic bulb (*Allium sativum*), White Willow bark (*Salix alba)*, Milk Thistle seed (*Silybum marianum*), Raspberry fruit (*Rubus idaeus*), Echinacea Purpurea plant extract (*Echinacea purpurea*), Echinacea Angustifolia root (*Echinacea angustifolia*), Black Walnut hull (*Juglans nigra*), Black Walnut leaf (*Juglans nigra*), Lavender oil (*Lavandula officinalis*), Oregano oil (*Origanum vulgare*), Galbanum oil (*Ferula galbaniflua*), Tea Tree oil (*Melaleuca alternifolia*), Fumitory aerial parts extract (*Fumaria officinalis*), Gentian Lutea root (*Gentiana lutea*).

Phospholipids (from Sunflower Seed lecithin)

Other Ingredients: Water, Glycerin, Ethanol, Vitamin E (as Tocofersolan and natural mixed tocopherols).

Overview

Biocidin® is a unique blend of 18 botanicals with wide-ranging biological actions, many of which are anti-inflammatory, and modulate both immune function and the balance of the intestinal microbiome. An essential element of Biocidin® is that it provides botanicals with multiple active constituents that have diverse and complementary mechanisms of action, in contrast to isolated components. Plants have evolved a richness in bioactive compounds and secondary metabolites, which provide for their own strategic defense and protection from both microbes and other threats, and which also may be lost with single compounds. For example, an antibacterial constituent such as berberine is substantially less effective without the synergism of Goldenseal's other components which target multi-drug resistance pumps, allowing for its intracellular accumulation.[1]

[1] Abreu AC, Coqueiro A, Sultan AR, et al. Looking to nature for a new concept in antimicrobial treatments: isoflavonoids from Cytisus striatus as antibiotic adjuvants against MRSA. Sci Rep. 2017 Jun 19;7(1):3777.

The botanicals in Biocidin® provide the terpenoids, phenolics, alkaloids and other active components of each plant that individually enhance a variety of functions, including induction of the Nrf2 antioxidant system, inhibition of multiple inflammatory regulators such as NF-κB and the NLRP3 inflammasome, as well as enhancement of both innate and cell-mediated immunity. Many of them have diverse strategies for targeting bacteria, fungi, viruses, and other pathogens, resulting in a shift to a more favorable microbiome while also providing enhanced immunity and integrity of the intestinal barrier, but their combined use presents an opportunity for a much broader synergistic effect. Biocidin® LSF utilizes Quicksilver liposomal technology, a natural phospholipid carrier designed to improve the bioavailability and stability of these botanicals.

Liposomal Technology

Liposomes are a type of vesicular carrier designed to improve the delivery of nutraceutical and botanical compounds, as well as medications, and have been extensively used in cosmetic and pharmaceutical industries for decades.[2,3] Liposomes are spherical, microscopic phospholipid carriers, consisting of an aqueous core entrapped by one or more (unilamellar vs. multilamellar) phospholipid membranes.[3] They have several attractive features, including high biocompatibility (e.g. lack of toxicity and/or immune activation), the ability to accommodate both water-soluble (in the aqueous core) and lipid-soluble (in the lamellae, or lipid layer) compounds, and to improve the uptake of poorly absorbed compounds.[3,4]

Many nutraceuticals and botanicals have poor or limited bioavailability, often related to their size, polarity, lack of stability, etc. Liposomes provide a vehicle to increase the absorption and availability of these compounds.[5] For some nutrients, liposomes may also allow for a higher peak plasma level; for example, in a small clinical trial, a liposomal vitamin C was shown to deliver a higher plasma peak compared to non-liposomal oral intake.[6] Plasma levels of quercetin, a flavonoid known to have poor absorption, were approximately 20-fold higher when incorporated into a phytosome (comprised of phospholipids similar to a liposome) compared to a standard form of quercetin.[7]

Biocidin® LSF utilizes the Quicksilver Delivery System, a natural phospholipid encapsulation technology made using sunflower seed lecithin, which provides vesicles of 100 nm or smaller particle size, classified as small unilamellar vesicles.[8] Smaller liposomes have been shown to be advantageous; liposomes with a smaller particle size have been associated with greater

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[2] Akbarzadeh A, Rezaei-Sadabady R, Davaran S, et al. Liposome: classification, preparation, and applications. Nanoscale Res Lett. 2013 Feb 22;8(1):102.
[3] Subramanian P. Lipid-Based Nanocarrier System for the Effective Delivery of Nutraceuticals. Molecules. 2021 Sep 10;26(18):5510.
[4] Din FU, Aman W, Ullah I, et al. Effective use of nanocarriers as drug delivery systems for the treatment of selected tumors. Int J Nanomedicine. 2017 Oct 5;12:7291-7309.
[5] McClements DJ, Li F, et al. The Nutraceutical Bioavailability Classification Scheme: Classifying Nutraceuticals According to Factors Limiting their Oral Bioavailability. Annu Rev Food Sci Technol. 2015;6:299-327.
[6] Davis JL, Paris HL, Beals JW, et al. Liposomal-encapsulated Ascorbic Acid: Influence on Vitamin C Bioavailability and Capacity to Protect Against Ischemia-Reperfusion Injury. Nutr Metab Insights. 2016 Jun 20;9:25-30.
[7] Riva A, Ronchi M, Petrangolini G, et al. Improved Oral Absorption of Quercetin from Quercetin Phytosome®, a New Delivery System Based on Food Grade Lecithin. Eur J Drug Metab Pharmacokinet. 2019 Apr;44(2):169-177.
[8] https://www.quicksilverscientific.com/quicksilver-delivery-systems Accessed 1/2022.

bioavailability than larger ones.[9] Liposomes can also improve bioavailability by increasing solubility in the GI tract, protecting molecules from first-pass metabolism (by stimulating intestinal lymphatic transport), and enhancing enterocyte-based transport.[3,10]

[9] Ong SG, Ming LC, Lee KS, et al. Influence of the Encapsulation Efficiency and Size of Liposome on the Oral Bioavailability of Griseofulvin-Loaded Liposomes. Pharmaceutics. 2016 Aug 26;8(3):25.
[10] Porter CJ, Trevaskis NL, Charman WN. Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. Nat Rev Drug Discov. 2007 Mar;6(3):231-48.

Bilberry extract (*Vaccinium myrtillus*)

Biological Actions:

Anti-inflammatory, antioxidant, modified microbiome, antibiofilm.

Scientific Evidence:

Bilberries are rich in biologically active compounds, including flavonols (such as quercetin and catechins) as well as phenols, particularly anthocyanins. Bilberries contain between 15-17 different anthocyanidins and anthocyanins (a glycosidic form of anthocyanidins), including delphinidins, cyanidins, petunidins, malvidins, and peonidins, shown to induce cellular protection against antioxidant stress, at least in part by upregulating the expression of antioxidant enzymes, including catalase and superoxide dismutase.[11,12] In the gastrointestinal tract anthocyanins improve permeability, in part, by providing protection against oxidative stress, but also by restoring tight junction integrity, and blocking pro-oxidant and inflammatory activity via several mechanisms, including mitigation of NF-κB activation, and by upregulating the expression of key tight junction proteins, including occludin, claudin-5, and zonula occludens-1.[13,14]

Anthocyanin extracts have demonstrated a bidirectional relationship with intestinal microbiota in several animal models, stimulating the production of beneficial bacteria and inhibiting pathogenic bacteria, which in turn increases the transformation of anthocyanins into more bioavailable and bioactive metabolites.[15] Bilberry anthocyanins have been shown to increase the diversity of bacterial species, including those that product short chain fatty acids (SCFAs), an important energy source for colonocytes but also an important modulator of mucosal immunity and inflammation.[16] The increase in the population of favorable species following anthocyanin administration, such as *Bifidibacterium* and *Akkermansia*, has also been associated with reduced intestinal inflammation as well as adipocyte metabolism.[17] Bilberry has also demonstrated antimicrobial action towards several pathogens *in vitro*, including *Streptococcus pneumoniaea, Neisseria meningitidis, Bacillus cereus*, *Citrobacter freundii*, *Enterococcus faecalis*, *Helicobacter*

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[11] Kuntz S, Kunz C, Herrmann J, et al. Anthocyanins from fruit juices improve the antioxidant status of healthy young female volunteers without affecting anti-inflammatory parameters: results from the randomised, double-blind, placebo-controlled, cross-over ANTHONIA (ANTHOcyanins in Nutrition Investigation Alliance) study. Br J Nutr. 2014 Sep 28;112(6):925-36.

[12] Khoo HE, Azlan A, Tang ST, et al. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food Nutr Res. 2017 Aug 13;61(1):1361779.

[13] Cremonini E, Daveri E, Mastaloudis A, et al. Anthocyanins protect the gastrointestinal tract from high fat diet-induced alterations in redox signaling, barrier integrity and dysbiosis. Redox Biol. 2019 Sep;26:101269.

[14] Dharmawansa KVS, Hoskin DW, Rupasinghe HPV. Chemopreventive Effect of Dietary Anthocyanins against Gastrointestinal Cancers: A Review of Recent Advances and Perspectives. Int J Mol Sci. 2020 Sep 8;21(18):6555.

[15] Ozdal T, Sela DA, Xiao J, et al. The Reciprocal Interactions between Polyphenols and Gut Microbiota and Effects on Bioaccessibility. Nutrients. 2016 Feb 6;8(2):78.

[16] Wang L, Jiang G, Jing N, et al. Bilberry anthocyanin extracts enhance anti-PD-L1 efficiency by modulating gut microbiota. Food Funct. 2020 Apr 30;11(4):3180-3190.

[17] Jayarathne S, Stull AJ, Park OH, et al. Protective Effects of Anthocyanins in Obesity-Associated Inflammation and Changes in Gut Microbiome. Mol Nutr Food Res. 2019 Oct;63(20):e1900149.

pylori, Salmonella, and *Staphylococcus aureus.*[18,19,20,21,22,23,24] The diversification of the microbiome and the biotransformation of anthocyanins have been proposed as likely mechanisms for the anti-inflammatory and other beneficial effects of these compounds.[25]

The anti-inflammatory effect of bilberry anthocyanins has also been observed in human studies; in an open pilot study in which participants with ulcerative colitis were given an anthocyanin-rich bilberry extract, colon biopsies revealed a modulation in inflammatory cytokines, including a reduction in IFN-γ, TNFα, and phosphorylated p65-NF-κB levels.[26] Clinical trials in humans suggest that the antioxidant and anti-inflammatory effects of bilberry anthocyanins improve both gastrointestinal and cardio-metabolic health. Trials of anthocyanin supplementation have been associated with improved liver enzymes and insulin sensitivity among participants with non-alcoholic fatty liver disease (NAFLD), as well as improved Mayo scores and reduced fecal calprotectin levels among participants with ulcerative colitis.[27,28] Although large clinical trials are lacking, trials of bilberry anthocyanins also suggest anti-atherogenicity among at-risk populations, e.g. several trials have shown improved cardiometabolic factors among participants with metabolic syndrome.[29,30,31,32]

Safety Summary:

Considered safe at the recommended dose.[11] No adverse effects expected during pregnancy and breastfeeding.[12]

Noni (*Morinda citrifolia*)

[18] Huttunen S, Toivanen M, Arkko S, et al. Inhibition activity of wild berry juice fractions against Streptococcus pneumoniae binding to human bronchial cells. Phytother Res. 2011 Jan;25(1):122-7.
[19] Toivanen M, Ryynänen A, Huttunen S, et al. Binding of Neisseria meningitidis pili to berry polyphenolic fractions. J Agric Food Chem. 2009 Apr 22;57(8):3120-7.
[20] Puupponen-Pimiä R, Nohynek L, Alakomi HL, et al. The action of berry phenolics against human intestinal pathogens. Biofactors. 2005;23(4):243-51.
[21] Burdulis D, Sarkinas A, Jakutiene I, et al. Comparative study of anthocyanin composition, antimicrobial and antioxidant activity in bilberry (Vaccinium myrtillus L.) and blueberry (Vaccinium corymbosum L.) fruits. Acta Pol Pharm. Jul-Aug 2009;66(4):399-408.
[22] Nohynek LJ, Alakomi HL, Kähkönen MP, et al. Berry phenolics: antimicrobial properties and mechanisms of action against severe human pathogens. Nutr Cancer. 2006;54(1):18-32.
[23] Puupponen-Pimiä R, Nohynek L, Alakomi HL, et al. Bioactive berry compounds-novel tools against human pathogens. Appl Microbiol Biotechnol. 2005 Apr;67(1):8-18.
[24] Chatterjee A, Yasmin T, Bagchi D, et al. Inhibition of Helicobacter pylori in vitro by various berry extracts, with enhanced susceptibility to clarithromycin. Mol Cell Biochem. 2004 Oct;265(1-2):19-26.
[25] Hair R, Sakaki JR, Chun OK. Anthocyanins, Microbiome and Health Benefits in Aging. Molecules. 2021 Jan 21;26(3):537.
[26] Roth S, Spalinger MR, Gottier C, et al. Bilberry-Derived Anthocyanins Modulate Cytokine Expression in the Intestine of Patients with Ulcerative Colitis. PLoS One. 2016 May 6;11(5):e0154817.
[27] Zhang PW, Chen FX, Li D, et al. A CONSORT-compliant, randomized, double-blind, placebo-controlled pilot trial of purified anthocyanin in patients with nonalcoholic fatty liver disease. Medicine (Baltimore). 2015 May;94(20):e758.
[28] Biedermann L, Mwinyi J, Scharl M, et al. Bilberry ingestion improves disease activity in mild to moderate ulcerative colitis - an open pilot study. J Crohns Colitis. 2013 May;7(4):271-9.
[29] Aboonabi A, Meyer RR, Gaiz A, et al. Anthocyanins in berries exhibited anti-atherogenicity and antiplatelet activities in a metabolic syndrome population. Nutr Res. 2020 Apr;76:82-93.
[30] de Mello VD, Lankinen MA, Lindström J, et al. Fasting serum hippuric acid is elevated after bilberry (Vaccinium myrtillus) consumption and associates with improvement of fasting glucose levels and insulin secretion in persons at high risk of developing type 2 diabetes. Mol Nutr Food Res. 2017 Sep;61(9).
[31] Chan SW, Tomlinson B. Effects of Bilberry Supplementation on Metabolic and Cardiovascular Disease Risk. Molecules. 2020 Apr 3;25(7):1653.
[32] Kolehmainen M, Mykkänen O, Kirjavainen PV, et al. Bilberries reduce low-grade inflammation in individuals with features of metabolic syndrome. Mol Nutr Food Res. 2012 Oct;56(10):1501-10.

Biological Actions:

Anti-inflammatory, antimicrobial, antioxidant.

Scientific Evidence:

To date, over 200 different compounds have been identified in the noni plant, including phenolics, flavonoids, anthraquinones, iridoids, lignans, and triterpenoids, which give rise to noni's potent antioxidant and anti-inflammatory properties.[33] The majority of these compounds have biological activity; iridoids have been shown to prevent the formation of advanced glycation end products (AGEs), with clinical trials among heavy smokers, who are known to have excessive oxidant exposure, demonstrating the iridoids in noni to be associated with a mitigation in both oxidative damage to DNA as well as cigarette-smoke induced dyslipidemia.[34,35,36] *In vitro* research has also shown that noni is highly effective at inhibiting hydroxyl radicals, known to cause oxidative damage to proteins, lipids, as well as DNA.[37]

As a natural anti-inflammatory agent, noni inhibits LPS-induced activation of several chemical mediators, including cyclooxygenase (COX)-1 and COX-2, nitric oxide and prostaglandins E_2 (PGE_2) in a dose dependent manner.[38] Damnacanthal, an anthraquinone found in noni, has been found to have immunomodulating and anti-inflammatory activity; it has been shown to suppress mast cell activation and allergic reactions by inhibiting the activation of several inflammatory mediators, including NF-κB and $p56^{lck}$ tyrosine kinase.[39,40] Noni also possesses immune stimulating properties, and based on *in vivo* and *in vitro* studies, enhances both cellular and humoral-mediated immunity.[41,42]

Noni has recently been found to influence glucose metabolism; animal studies suggest that this may occur via several mechanisms, including an increased sensitivity to insulin via an inhibition of protein tyrosine phosphatase 1B (PTP1B), a known inducer of insulin resistance, as well as through inhibition of forkhead box O (FoxO1) transcription, a key regulator of

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[33] Inada AC, Figueiredo PS, Santos-Eichler RAD, et al. Morinda citrifolia Linn. (Noni) and Its Potential in Obesity-Related Metabolic Dysfunction. Nutrients. 2017 May 25;9(6):540.
[34] West BJ, Deng S, Uwaya A, et al. Iridoids are natural glycation inhibitors. Glycoconj J. 2016 Aug;33(4):671-81.
[35] Wang MY, Peng L, Weidenbacher-Hoper V, et al. Noni juice improves serum lipid profiles and other risk markers in cigarette smokers. ScientificWorldJournal. 2012;2012:594657.
[36] Wang MY, Peng L, Jensen CJ, et al. Noni juice reduces lipid peroxidation-derived DNA adducts in heavy smokers. Food Sci Nutr. 2013 Mar;1(2):141-9.
[37] Serafini MR, Santos RC, Guimaraes AG, et al. Morinda citrifolia Linn leaf extract possesses antioxidant activities and reduces nociceptive behavior and leukocyte migration. J Med Food. Oct 2011;14(10):1159- 1166.
[38] Dussaussoy E, Brat P, Bony E, et al. Characterization, anti-oxidative and anti-inflammatory effects of Costa Rican noni juice (Morinda citrifolia L.). J Ethnopharmacol. Jan 7 2011;133(1):108-115.
[39] Garcia-Vilas JA, Medina MA, Melo FR, et al. Damnacanthal inhibits IgE receptor-mediated activation of mast cells. Mol Immunol. 2015 May;65(1):86-93.
[40] Kim MH, Jeong HJ. Damnacanthal inhibits the NF-κB/RIP-2/caspase-1 signal pathway by inhibiting p56lck tyrosine kinase. Immunopharmacol Immunotoxicol. 2014 Oct;36(5):355-63.
[41] Nayak S, Mengi S. Immunostimulant activity of noni (Morinda citrifolia) on T and B lymphocytes. Pharm Biol. Jul 2010;48(7):724-731.
[42] Lohani M, Majrashi M, Govindarajulu M, et al. Immunomodulatory actions of a Polynesian herb Noni (Morinda citrifolia) and its clinical applications. Complement Ther Med. 2019 Dec;47:102206.

gluconeogenesis.[43,44] Human clinical trials are sparse but have demonstrated a hypoglycemic and anti-inflammatory effect of noni among participants with type 2 diabetes.[45]

Several active compounds in noni, including aucubin, L-asperuloside and alizarin as well as the phenolics 5,15-dimethylmorindol, ferulic acid, p-hydroxycinamic acid, methyl 4-hydroxybenzoate, methyl ferulate, and methyl 4-hydroxycinnamate have demonstrated antibacterial activity against a number of pathogens including *Pseudomonas aeruginosa*, *Proteus morganii*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* and *Shigella*.[46,47] Noni has also been shown to inhibit the activity of enterohemorrhagic *Escherichia coli* (0157) and *Helicobacter pylori*.[48,49]

Traditionally noni was used for tuberculosis infections, which has now been substantiated by *in vitro* studies indicating noni is nearly as effective as Rifampin (with inhibition rates of 89% and 97% respectively).[50,51] Noni has demonstrated antifungal activity against *Candida albicans* in a dose dependent manner.[52,53] Aqueous extracts of noni may also help protect against the conversion of cellular *Candida albicans* into the hyphenated or filamentous form of the yeast. Germ tube formation or hyphenation from blastoconidia by *Candida* species is thought to be a virulence factor in their pathogenesis. Similarly, noni has been shown to inhibit the germination of spores from the filamentous fungi *Aspergillus nidulans*.[54]

Safety Summary:

No known warnings, precautions or contraindications at the dose recommended.[13,55] No adverse effects expected during pregnancy and breastfeeding.[13,56]

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[43] Nerurkar PV, Nishioka A, Eck PO, et al. Regulation of glucose metabolism via hepatic forkhead transcription factor 1 (FoxO1) by Morinda citrifolia (noni) in high-fat diet-induced obese mice. Br J Nutr. 2012 Jul;108(2):218-228.

[44] Nerurkar PV, Hwang PW, Saksa E. Anti-Diabetic Potential of Noni: The Yin and the Yang. Molecules. 2015 Sep 25;20(10):17684-719.

[45] Algenstaedt P, Stumpenhagen A, Westendorf J. The Effect of Morinda citrifolia L. Fruit Juice on the Blood Sugar Level and Other Serum Parameters in Patients with Diabetes Type 2. Evid Based Complement Alternat Med. 2018 Aug 6;2018:3565427.

[46] Zhang WM, Wang W, Zhang JJ, et al. Antibacterial Constituents of Hainan Morinda citrifolia (Noni) Leaves. J Food Sci. 2016 May;81(5):M1192-6.

[47] Wang MY, West BJ, Jensen CJ, et al. Morinda citrifolia (Noni): a literature review and recent advances in Noni research. Acta Pharmacol Sin. Dec 2002;23(12):1127-1141.

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[50] Mauliku, N. E., Hendro, W., Saputo, et al. Anti-tubercular activity of extract and coumponds of noni (Morinda citrifolia Linn). International Journal of Pharmacy and Pharmaceutical Sciences. 2017; 9(12), 105–109.

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[52] Jainkittivong A, Butsarakamruha T, Langlais RP. Antifungal activity of Morinda citrifolia fruit extract against Candida albicans. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009 Sep;108(3):394-8.

[53] Barani K, Manipal S, Prabu D, et al. Anti-fungal activity of Morinda citrifolia (noni) extracts against Candida albicans: an in vitro study. Indian J Dent Res. 2014 Mar-Apr;25(2):188-90.

[54] Banerjee S, Johnson AD, Csiszar K, et al. An extract of Morinda citrifolia interferes with the serum-induced formation of filamentous structures in Candida albicans and inhibits germination of Aspergillus nidulans. Am J Chin Med. 2006;34(3):503-9.

[55] West BJ, White LD, Jensen CJ, Palu AK. A double-blind clinical safety study of noni fruit juice. Pac Health Dialog. 2009 Nov;15(2):21-32.

[56] Wang MY, Hurn J, Peng L, et al. A multigeneration reproductive and developmental safety evaluation of authentic Morinda citrifolia (noni) juice. J Toxicol Sci. 2011 Jan;36(1):81-5.

Milk Thistle (*Silybum marianum*)

Biological Actions:

Antimicrobial, antioxidant, anti-inflammatory, choleretic, hepatoprotective, antibiofilm.

Scientific Evidence:

Milk thistle is rich in flavonolignans which are composed of silybin A and silybin B (diastereoisomers), silydianin, silychristin and diastereoisomers isosilybin A and isosilybin B. These polyphenolic molecules are collectively referred to as silymarin.[11] Research has shown that the flavonolignans from milk thistle possess potent antibacterial activity against Gram-positive bacteria, but no antimicrobial activity against Gram-negative bacteria.[57] In addition to direct anti-bacterial action, silymarin has also been shown to inhibit the adherence and formation of bacterial biofilms.[58] Silymarin also inhibits the growth of multiple species of *Candida*, destabilizing mature biofilms and inhibiting the secretion of phospholipases and proteinases, an important determinant of fungal virulence.[59]

Silibinin (an equal extract of silybin A and silybin B) has demonstrated antibacterial activity against methicillin-resistant strains of *Staphylococcus aureus*.[11,60] When silibinin was combined with the antibiotics oxicillin or ampicillin there was a more than four-fold reduction in the minimum inhibitory bactericidal concentrations. Based on *in vitro* research, silibinin's antimicrobial properties are due to its ability to inhibit ribonucleic acid (RNA) and protein synthesis of Gram-positive organisms (as opposed to attacking the bacterial membrane).[61] Ethanol extracts of silibin have also demonstrated *in vitro* antibacterial activity against *Campylobacter jejuni*, and the purified flavonolignan dehydroisosilybin has inhibited the *in vitro* growth of two species of *Leishmania* parasites.[62,63] Silymarin has also demonstrated antiviral activity against influenza A/PR/8/34 virus when compared with the pharmaceutical agent Oseltamivir (98% vs. 52% respectively).[64] Its ability to suppress cellular inflammation, including inhibition of mTOR, may partly explain its immunomodulating effects.[65,66]

Silibinin has also demonstrated antioxidant and anti-inflammatory properties in LPS-stimulated human monocytes through an inhibitory effect on hydrogen peroxide release and tumor

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[57] Lee DG, Kim HK, Park Y, Park SC, Woo ER, Jeong HG, Hahm KS. Gram-positive bacteria specific properties of silybin derived from Silybum marianum. Arch Pharm Res. 2003 Aug;26(8):597-600.
[58] Evren E, Yurtcu E. In vitro effects on biofilm viability and antibacterial and antiadherent activities of silymarin. Folia Microbiol (Praha). 2015 Jul;60(4):351-6.
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[61] Wang X, Zhang Z, Wu SC. Health Benefits of Silybum marianum: Phytochemistry, Pharmacology, and Applications. J Agric Food Chem. 2020 Oct 21;68(42):11644-11664.
[62] Cwikla C, Schmidt K, Matthias A, et al. Investigations into the antibacterial activities of phytotherapeutics against Helicobacter pylori and Campylobacter jejuni. Phytother Res. 2010 May;24(5):649-56.
[63] Olías-Molero AI, Jiménez-Antón MD, Biedermann D, et al. In-Vitro Activity of Silybin and Related Flavonolignans against Leishmania infantum and L. donovani. Molecules. 2018 Jun 27;23(7):1560.
[64] Song JH, Choi HJ. Silymarin efficacy against influenza A virus replication. Phytomedicine. Jul 15 2011;18(10):832-835.
[65] Lovelace ES, Wagoner J, MacDonald J, et al. Silymarin Suppresses Cellular Inflammation By Inducing Reparative Stress Signaling. J Nat Prod. 2015 Aug 28;78(8):1990-2000.
[66] Lovelace ES, Maurice NJ, Miller HW, et al. Silymarin suppresses basal and stimulus-induced activation, exhaustion, differentiation, and inflammatory markers in primary human immune cells. PLoS One. 2017 Feb 3;12(2):e0171139.

necrosis-alpha (TNFα) production.[67] Silibin's anti-inflammatory targets may also include the NLRP3 inflammasome, with several models citing an inhibition NF-κB and NLRP3 signaling pathways.[68,69,70] The involvement of the NLRP3 inflammasome in multiple inflammatory conditions, including NAFLD and diabetes, suggests a mechanism of potential benefit for milk thistle, with growing clinical support. A review of eight randomized trials of participants with NAFLD found a significant reduction in transaminase levels with silymarin use, and a recent meta-analysis points to a general improvement in the glycemic profile of participants with glucose or lipid abnormalities.[71,72] Silymarin supplementation was also associated with an improvement in antioxidant levels and total antioxidant capacity, as well as reduced inflammation in a triple-blind trial of participants with diabetes.[73]

Milk thistle has several hepatoprotective effects, including the upregulation of thioredoxin and sirtuins, as well as the bile salt export pump, in addition to having an anti-fibrotic effect on stellate cells in myofibroblasts.[74,75] Silymarin provides protection against multiple exogenous toxins, mediated in part by an activation of nuclear factor erythroid 2-related factor 2 (Nrf2), a key regulator of cellular antioxidant enzymes.[76,77] The upregulation of Nrf2 increases expression of multiple antioxidant and detoxification enzymes, which have been associated with improved intestinal immune function, barrier integrity, and reduced mucosal injury and inflammation.[78,79]

Safety Summary:

Contraindicated in persons allergic to plants from the Compositae (aka Asteraceae) family. No other known warnings, precautions, or contraindications. No adverse effects expected during pregnancy and breastfeeding.[80,81,82]

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[67] Bannwart CF, Peracoli JC, Nakaira-Takahagi E, et al. Inhibitory effect of silibinin on tumour necrosis factor-alpha and hydrogen peroxide production by human monocytes. Nat Prod Res. Nov 2010;24(18):1747-1757.
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Echinacea (*Echinacea purpurea & Echinacea angustifolia*)

Biological Actions:

Anti-inflammatory, antifungal, antiviral, immune modulator.

Scientific Evidence:

Echinacea possesses both anti-inflammatory and immuno-stimulating properties.[83] Alkylamides, one of the active constituents of echinacea, are thought to be responsible for the herb's anti-inflammatory activity. The alkylamides have been found to modulate production of the inflammatory mediators TNFα and PGE_2, and to inhibit mast cell degranulation.[84]

Echinacea also modulates both innate and adaptive immune responses, with models revealing greater T cell proliferation and increased activity of both macrophages and natural killer cells.[85,86] Several specific immune effects have been attributed to components of Echinacea; a polysaccharide enriched extract of *Echinacea purpurea* has been shown to activate macrophages, polarizing them toward the M1 phenotype, associated with enhanced bactericidal and phagocytic activity.[87] N-alkylamides from Echinacea have also been shown to synergistically activate the cannabinoid receptor type-2 (CB2) and stimulate production of the anti-inflammatory cytokine IL-10.[88] Proteobacteria which colonize Echinacea have also been found to enhance immune function by activating macrophages, suggesting that Echinacea has a probiotic-like effect.[89,90,91] Activation of the Toll-like receptor 2 and 4 pathways by bacterial lipoproteins and lipopolysaccharides may provide the mechanism for macrophage and NK cell activation.[92] Echinacea has also been shown to prevent the decrease in mucosal immunity following exercise; in a controlled clinical trial Echinacea was found to prevent the drop in SIgA secretion

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post-exercise and reduce the duration of upper respiratory tract infections during a 4-week intervention.[93]

Echinacea has demonstrated *in vitro* antimicrobial activity against many common pathogens including *Streptococcus pyogenes*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Propionibacterium acnes*, *Legionella pneumophila*, *Clostridium difficile* and *Candida albicans*.[94,95]

As a natural antiviral agent, Echinacea has demonstrated efficacy against many viruses, including influenza viruses (A and B strains), respiratory syncytial virus, rhinovirus, herpes simplex virus (HSV-1), calicivirus and coronavirus.[99,96,97,98] Based upon *in vitro* research, possible antiviral mechanisms of action for echinacea include pro-inflammatory cytokine inhibition (specifically IL-6 and IL-8) and upregulation of inducible nitric oxide synthase (iNOS).[99,100,101,102] Controlled clinical trials suggest both a reduction in upper respiratory virus infections and a subsequent reduction in the need for antibiotics as well, an effect that is likely dependent on the preparation used.[103,104,105]

Safety Summary:

Contraindicated in persons allergic to plants from the Compositae aka (Asteraceae family).[12] Exercise caution with patients taking immunosuppressant medications (short term use only).[12] No other known warnings, precautions, or contraindications.[106] No adverse effects expected during pregnancy and breastfeeding.[12,111,107]

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Goldenseal (*Hydrastis canadensis*)

Biological Actions:

Antibacterial, antihistamine, anti-inflammatory, antiviral, antifungal, antiprotozoal, cardio-metabolic aid, antibiofilm.

Scientific Evidence:

Goldenseal root contains multiple alkaloids, the most abundant of which is berberine, as well as canadine, canadaline, and hydrastine. Both *in vivo* and *in vitro* studies have revealed that berberine possesses antimicrobial activity against bacteria, fungi and parasites.[12,113] Goldenseal leaves are also rich in flavonoids; two of which (6,8-di-C-methylluteolin 7-methyl ether and 6-C-methylluteolin 7-methyl ether) have demonstrated antibacterial activity against the oral pathogens *Streptococcus mutans* and *Fusobacterium nucleatum*, while others (specifically sideroxylon, 8-desmethyl-sideroxylon and 6-desmethyl-sideroxylin) don't appear directly antibacterial, but instead enhance the action of berberine by acting as efflux pump inhibitors.[108] It should be noted that one of the major mechanisms by which bacteria become resistant to antibiotics is by overexpression of efflux pumps, which are also known as multidrug resistance pumps.[109] In one *in vitro* study, inhibition of the efflux pump allowed a much greater intracellular concentration of berberine, potentiating its antibiotic activity 500-fold against some organisms, indicating the importance of the synergistic interactions among goldenseal's constituents.[110]

The combined effects of the active constituents in goldenseal make this herb a potent antimicrobial agent for a number of Gram-positive and Gram-negative organisms including methicillin-resistant *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus sanguis*, *Pseudomonas aeruginosa*, *Mycoplasma mycoides capri*, *Escherichia coli*, *Neisseria gonorrhoeae* isolates (including antibiotic-resistant strains), *Campylobacter jejuni, Vibrio cholera* and *Helicobacter pylori*.[111,112,113,114,115] Berberine, as part of quadruple therapy, has been found to be non-inferior to bismuth for the eradication of *Helicobacter* pylori in a phase 4 trial.[116] One of the key mechanisms by which goldenseal inhibits microbial growth is through quenching of the *agr*

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quorum sensing (QS) system.[117] The QS system is bacterial cell-to-cell communication that controls gene expression and influences many physiological processes including bioluminescence, sporulation, competence, antibiotic production, biofilm formation and virulence factor secretion.[118] Berberine specifically has been shown to disrupt biofilms in *Salmonella typhimurium*, at least in part by reducing the number of type I fimbriae, an important virulence factor among members of the *Enterobacteriaceae* family.[119]

Berberine has demonstrated antifungal activity against the non-albicans *Candida* species (specifically *Candida krusei*, *Candida Kefyr*, *Candida glabrata*, *Candida tropicalis* and *Candida parapsilosis).* When combined with the antimycotic drugs miconazole or fluconazole, berberine was able to reduce biofilm formation of pathogenic *C. albicans*.[120] *In vitro* data shows antifungal activity against not just *Candida*, but also *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Trichophyton mentagrophytes*, *Microsporum canis*, *Trichophyton rubrum*, *Epidermophyton floccosum*, and *Microsporum gypseum*.[113] One analysis revealed a probable mechanism of action to be the disruption of both plasma and mitochondrial fungal membranes, as well as disruption of fungal biofilms.[121] *In vitro* studies have shown that berberine possesses significant antimicrobial activity against a number of protozoans including *Blastocystis hominis*, *Giardia lamblia*, *Entamoeba histolytica*, *Trichomonas vaginalis* and *Leishmania donovani*.[113] Multiple mechanisms of action have been documented for berberine's anti-protozoal activity, including a direct effect, via oxidative bursts in parasites, as well as an indirect effect, via modulation of the mitogen activated protein kinase (MAPK) cascade.[122]

Berberine has also been shown to inhibit the growth of several viruses including cytomegalovirus, human papillomavirus (HPV), CHIKV, HSV-I and human H1N1 strains of influenza A.[123] One mechanism for its anti-viral activity is the inhibition of the MAPK pathway, a common viral target to manipulate cellular functions. Berberine has also demonstrated an ability to reduce inflammation triggered by viral infections, mediated in part by activation of AMP-activated protein kinase (AMPK), and inhibition of NF-κB.[124] Additionally, both *in vitro* and *in vivo* models suggest an antihistamine effect of berberine, in part mediated via mast cell

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stabilization as well as enhanced function and quantity of T_{reg} cells.[125,126] Berberine also has the ability to upregulate the Nrf2 signaling pathway, a gatekeeper for cellular antioxidant defense.[127]

In addition to its many antimicrobial actions, berberine has been shown to broadly alter microbiome composition and functionality. In one animal model this was associated with a decrease in the gut microbiota metabolite trimethylamine (TMA, a marker for atherosclerosis) following a decrease in the population of pathogenic bacteria and an increase in beneficial bacteria.[128] The shift in the microbiome population frequencies is comparable to a probiotic-like effect that may help to explain favorable cardiometabolic effects, (often attributed to an increased production of short chain fatty acids as well as reduced levels of LPS) including improved lipid metabolism and insulin sensitivity.[129] For example, in one animal model berberine was shown to reduce the activity of *Clostridium* cluster XIVa and IV. This subsequently reduced the production of bile salt hydrolase, an inhibitor of taurocholic acid (TCA) synthesis. An increase in TCA activates intestinal farnesoid X receptor (FXR), involved in the metabolism of bile acids, lipids, and glucose. A parallel effect is observed with an increase in the population of butyrate-producing bacteria, which can also reduce serum lipids and glucose.[130,131] Another mechanism was recently revealed in a clinical trial of participants with NAFLD; berberine was shown to influence sphingolipid metabolism, including a decrease in serum ceramides. This was correlated with improvements in glucose and lipid profiles compared to a lifestyle intervention, as well as a reduction in hepatic fat content among participants receiving berberine.[132]

In a related clinical trial, this metabolic effect was marked by changes in hemoglobin A1c (HbA1c) among over 400 subjects with diabetes participating in a randomized and controlled parallel 4-arm trial, supplemented with either berberine, probiotics, berberine and probiotics, or placebo. Both groups receiving berberine had significantly greater reductions in HbA1c; metagenomic and metabolomic analysis revealed significant shifts in the microbiome, and a correlation between the HbA1c decrease and a decrease in the population of *Ruminococcus bromii*, a species known to increase the biotransformation of the bile acid deoxycholic acid species (DCAs).[133] Cardiometabolic changes, including more favorable glucose and lipid profiles, were recently supported by a systematic review and meta-analysis of randomized controlled trials.[134]

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Safety Summary:

Exercise caution in patients with kidney disease.[13] No other known warnings, precautions or contraindications at the dose recommended.[12] Contraindicated during pregnancy in therapeutic doses.[13] Discouraged during breastfeeding in therapeutic doses.[11]

Shiitake mushroom (*Lentinula edodes*)

Biological Actions:

Antibacterial, antifungal, antioxidant, immune modulating, antibiofilm.

Scientific Evidence:

Shiitake mushroom contains many compounds of interest for their immunomodulating properties. Among these are the beta-glucan lentinan, the activated hexose correlated compound (AHCC), and the polysaccharide arabinoxylan. Lentinan has been found to increase the ratio of the Th1 to Th2 response, and in human trials, it has increased the number of B cells and quality of life among healthy adults.[135,136,137] In an animal model, it also reduced intestinal inflammation via inhibition of IL-8 expression, thereby inhibiting NF-κB activation.[138] Lentinan has also up-regulated expression of the p53-dependent signaling pathway, as well as other immune responses that maintain cellular integrity.[139] Among young healthy adults, a randomized clinical trial also found a variety of benefits among those participants consuming shiitake mushrooms daily versus controls; increases in specific immune markers, including sIgA levels, γδ-T and natural killer T cell proliferation, as well as a reduction in C-reactive protein and a more favorable cytokine profile suggest broad anti-inflammatory and immune modulating effects.[140]

Activated hexose correlated compound (AHCC), a standardized extract of cultured shiitake, has been shown to upregulate several immune pathways in animal models, marked by increased production of IFN-γ by T cells and enhanced NK cell activity, which may explain its observed anti-viral properties.[141] AHCC has been shown to prime the TL-2 and TL-4 receptors in the

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[135] Gaullier JM, Sleboda J, Øfjord ES, et al. Supplementation with a soluble β-glucan exported from Shiitake medicinal mushroom, Lentinus edodes (Berk.) singer mycelium: a crossover, placebo-controlled study in healthy elderly. Int J Med Mushrooms. 2011;13(4):319-26.

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intestine and increase the number of IgA+ plasma cells in an animal model.[142] In a small randomized and controlled human trial, it also improved antibody titers against influenza B when given immediately following vaccination.[143] Rice bran arabinoxylan compound (RBAC), created by fermenting rice bran with shiitake mushroom, contains the polysaccharide arabinoxylan. This extract has been found to improve macrophage phagocytosis and enhance the anti-bacterial activity of neutrophils and monocytes.[144] In a small, randomized trial, 63% of participants with irritable bowel syndrome given Biobran (arabinoxylan compound derived from rice bran fermentation) had subjective improvement, vs. 30% of those given placebo, as well as significant increases in NK cell activity and reductions in C-reactive protein.[145]

Based on *in vitro* research, shiitake mushroom has demonstrated antibacterial activity against a number of organisms including *Bacillus* sp., *Escherichia coli*, *Enterobacter spp., Klebsiella* sp., *Serratia* sp., *Pseudomonas aeruginosa*, *Listeria monocytogenes, Salmonella poona, Cupriavidus* sp., *Staphylococcus* sp. (including methicillin-resistant *Staphylococcus aureus* (MRSA)), *Staphylococcus epidermidis*, *Streptococcus pyogenes* and *Enterococcus faecalis*.[146,147,148,149] One mechanism of action for this antibacterial activity is protein leakage following destruction of the bacterial cell membrane.[150]

Shiitake mushroom extracts have also demonstrated anti-biofilm activity against oral pathogens, including *Streptococcus mutans* and *Actinomyces naeslundii.*[151] Other compounds in shiitake extract have also demonstrated the ability to disrupt bacterial biofilms, including erythritol, adenosine, carvacrol, and may help to support oral health, in part, by an anti-cariogenic effect.[152,153]

Shiitake has also displayed antifungal activity against the following microbes; *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Aspergillus fumigatus*, *Aspergillus niger*, and *Scedosporium apiospermum*.[153] Unlike antibiotics, the probiotic strains *Bifidobacterium and Lactobacillus spp* were not affected by the antimicrobial activities of shiitake mushroom.[156]

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Safety Summary:

Considered safe and well tolerated at doses of up to 2.5mg Lentinex per day for 6 weeks.[142] Doses of 9 grams per day of liquid AHCC have also been trialed for two weeks in healthy adults with no changes in blood chemistry markers or significant adverse events.[154] No adverse effects expected during pregnancy and breastfeeding at the dose recommended.[155]

White willow bark (*Salix alba*)

Biological Actions: Analgesic, anti-inflammatory.

Scientific Evidence:

The key active constituents of white willow bark are comprised of phenolic glycosides including the salicylates salicortin and salicin.[11] However, an analysis of white willow bark revealed at least 16 other important compounds, including the flavonoids naringenin and isosalipurpuroside (also known as eriodictyol), condensed tannins, catechin, amelopsin, taxifolin, 7-O-methyltaxifolin-3'-O-glucoside, and 7-O-methyltaxifolin.[156,157,158,159] Initially it was thought that salicin (converted to salicylic acid *in vivo*) was responsible for the anti-inflammatory effects of this herb.[164] More recent evidence suggests that the potent anti-inflammatory effect is derived from the sum total of the biologically active components, given white willow bark's effects are much broader acting than non-steroidal anti-inflammatory drugs (NSAIDs) which contain acetylsalicylic acid.[160] Unlike NSAIDs, white willow bark is not associated with unwanted side effects of gastric erosion.[165]

The synergistic effect of the salicylates, flavonoids and tannins found in white willow bark have been shown to inhibit COX-2 and subsequent generation of free radicals by converting arachidonic acid to prostaglandins.[161] *In vitro* studies assessing LPS activated monocytes show that *Salix alba* is able to block nitric oxide release and reduce IL-6 and TNF α production.[164,162] While the underlying mechanisms have not been fully elucidated, white willow bark appears to induce monocyte apoptosis and block NF-κB activation.[164,165] This multifactorial effect is thought to be an innate protective mechanism to control local and systemic inflammatory responses in the

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[154] Spierings EL, Fujii H, Sun B, et al. A Phase I study of the safety of the nutritional supplement, active hexose correlated compound, AHCC, in healthy volunteers. J Nutr Sci Vitaminol (Tokyo). 2007 Dec;53(6):536-9.
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[156] Pobłocka-Olech L, van Nederkassel AM, Vander Heyden Y, et al. Chromatographic analysis of salicylic compounds in different species of the genus Salix. J Sep Sci. 2007 Nov;30(17):2958-66.
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[158] Bonaterra GA, Kelber O, Weiser D, et al. In vitro anti-proliferative effects of the willow bark extract STW 33-I. Arzneimittelforschung. 2010;60(6):330-5.
[159] Agnolet S, Wiese S, Verpoorte R, et al. Comprehensive analysis of commercial willow bark extracts by new technology platform: combined use of metabolomics, high-performance liquid chromatography-solid-phase extraction-nuclear magnetic resonance spectroscopy and high-resolution radical scavenging assay. J Chromatogr A. 2012 Nov 2;1262:130-7.
[160] Shara M, Stohs SJ. Efficacy and Safety of White Willow Bark (Salix alba) Extracts. Phytother Res. 2015 Aug;29(8):1112-6.
[161] Fiebich BL, Chrubasik S. Effects of an ethanolic salix extract on the release of selected inflammatory mediators in vitro. Phytomedicine. 2004 Feb;11(2-3):135-8.
[162] Drummond EM, Harbourne N, Marete E, et al. Inhibition of proinflammatory biomarkers in THP1 macrophages by polyphenols derived from chamomile, meadowsweet and willow bark. Phytother Res. 2013 Apr;27(4):588-94.

body.[164] An antioxidant effect was also recently documented for salicin specifically, mediated in part by activation of the PI3K/Akt/GSK3β pathway, which plays a role in cellular protection, particularly against ischemic injury.[163]

Safety Summary:

Contraindicated in people with salicylate sensitivity.[167] White willow bark contains salicylic acid, the active constituent in aspirin. Although dosing with aspirin during viral infection is contraindicated in children under 16, the levels in Biocidin are very small. There have been no reported cases of Reye's Syndrome with the use of Biocidin. If known metabolic defects are present, it may be prudent to avoid all salicylic acid containing products - as determined by practitioner discretion. No other known warnings, precautions or contraindications at the dose recommended.[13] Should be avoided during pregnancy.[164] Discouraged during breastfeeding in therapeutic doses.

Garlic (*Allium sativum*)

Biological Actions:

Anthelmintic, anti-inflammatory, antimicrobial, antioxidant, antibiofilm.

Scientific Evidence:

The most biologically active constituent of garlic is allicin (S-(2-propenyl)-2-propene-1-sulfinothioate), which is formed when the herb is crushed and alliinase (an enzyme from the bundle sheath cells) combines with the substrate alliin (S-allyl-L-cysteine sulfoxide).[165] Bulbs of garlic contain hundreds of other phytochemicals, including many sulfur containing compounds, ajoenes (E-ajoene, Z-ajoene), thiosulfinates (allicin), vinyldithiins (2-vinyl-(4H) -1,3-dithiin, 3-vinyl-(4H)-1,2-dithiin), sulfides (diallyl disulfide (DADS), diallyl sulfide (DAS), diallyl trisulfide (DATS)), N-acetylcysteine (NAC), S-allyl-cysteine (SAC), and others.[172]

Much of the antimicrobial activity of garlic has been attributed to allicin activity, and includes both Gram-positive and Gram-negative microorganisms, as well as antibiotic-resistant bacteria, including *Shigella*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Salmonella enterica*, *Klebsiella aerogenes*, *Vibrio*, *Mycobacteria*, *Proteus vulgaris*, and *Enterococcus faecalis.*[172,166] The antimicrobial activity of allicin has been partly attributed to the S-allylmercapto modification of thiol-containing proteins in bacteria, such as glutathione, leading to either necrosis or apoptosis.[167] However, allicin is a

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[163] Park JH, Lee TK, Kim DW, et al. Neuroprotective Effects of Salicin in a Gerbil Model of Transient Forebrain Ischemia by Attenuating Oxidative Stress and Activating PI3K/Akt/GSK3β Pathway. Antioxidants (Basel). 2021 Apr 20;10(4):629.

[164] Oketch-Rabah HA, Marles RJ, Jordan SA, et al. United States Pharmacopeia Safety Review of Willow Bark. Planta Med. 2019 Nov;85(16):1192-1202.

[165] El-Saber Batiha G, Magdy Beshbishy A, G Wasef L, et al. Chemical Constituents and Pharmacological Activities of Garlic (Allium sativum L.): A Review. Nutrients. 2020 Mar 24;12(3):872.

[166] Wallock-Richards D, Doherty CJ, et al. Garlic revisited: antimicrobial activity of allicin-containing garlic extracts against Burkholderia cepacia complex. PLoS One. 2014 Dec 1;9(12):e112726.

[167] Müller A, Eller J, Albrecht F, et al. Allicin Induces Thiol Stress in Bacteria through S-Allylmercapto Modification of Protein Cysteines. J Biol Chem. 2016 May 27;291(22):11477-90.

very unstable compound, and thus unlikely to be the only antimicrobial component of garlic *in vivo*.

Both *in vitro* and *in vivo* studies have identified the two ajoenes (Z and E) as components of garlic that are able to inhibit virulence genes controlled by quorum sensing (QS) systems, virulence factors that are also of critical importance to the formation of biofilms and antibiotic resistance.[168,169] Ajoenes have shown antimicrobial activity against a variety of both Gram-negative and Gram-positive bacteria, and may play a role in the effectiveness of garlic against a number of pathogens with multiple drug-resistances.[170,171] DAS has also been found to inhibit the transcription of virulence genes in *Pseudomonas aeruginosa* which are regulated by the QS system, as well as most of the key genes in the QS system, indicating that multiple components within garlic may target this mechanism.[172] Furthermore, QS inhibitors have demonstrated a synergistic effect when combined with antibiotics. Based on *in vitro* research, the addition of ajoene to a *Pseudomonas* biofilm plus tobramycin killed more than 90% of the bacteria (compared with no effect when tobramycin was tested in isolation).[175] Allicin also has an extensive number of bacterial and fungal pathogens for which it acts synergistically against when coupled with other antibiotics.[173] Research shows that garlic has a temporal effect on commensal flora – when initially exposed to the herb, probiotic strains such as *Lactobacillus* are transiently inhibited, followed by a resurgence of growth with bacterial counts comparable to levels preceding garlic intervention.[174]

Garlic is also known to have antifungal activity against a variety of organisms, including *Candida*, *Torulopsis*, *Trichophyton*, *Cryptococcus*, *Aspergillus*, *Trichosporon*, and *Rhodotorula* species. Garlic has been shown to target fungal cell walls, and cause irreversible structural changes in the fungal cells, leading to cell death.[172] Anthelmintic activity against *Haemonchus contortus*, *Trichuris muris* and *Angiostrongylus cantonensis* has also been demonstrated with various garlic extracts, and allicin, ajoenes, and diallyl trisulfide have all shown activity against a variety of parasites.[172]

In addition to its broad anti-microbial effects, garlic has also been found to have anti-inflammatory, antioxidant, and cardiometabolic effects. Allicin specifically has been shown to active the Nrf2 pathway, attenuate an LPS-induced inflammatory response, and limit reactive oxygen species, mitochondrial dysfunction, and lipid peroxidation among cultured human

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[168] Jakobsen TH, van Gennip M, Phipps RK, et al. Ajoene, a sulfur-rich molecule from garlic, inhibits genes controlled by quorum sensing. Antimicrob Agents Chemother. 2012 May;56(5):2314-25.
[169] Nadell CD, Xavier JB, Levin SA, et al. The evolution of quorum sensing in bacterial biofilms. PLoS Biol. 2008 Jan;6(1):e14.
[170] Naganawa R, Iwata N, Ishikawa K, et al. Inhibition of microbial growth by ajoene, a sulfur-containing compound derived from garlic. Appl Environ Microbiol. 1996 Nov;62(11):4238-42.
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[173] Choo S, Chin VK, Wong EH, et al. Review: antimicrobial properties of allicin used alone or in combination with other medications. Folia Microbiol (Praha). 2020 Jun;65(3):451-465.
[174] Filocamo A, Nueno-Palop C, Bisignano C, et al. Effect of garlic powder on the growth of commensal bacteria from the gastrointestinal tract. Phytomedicine. 2012 Jun 15;19(8-9):707-11.

umbilical vein endothelial cells (HUVECs).[175] It has also been found to prevent endothelial injury resulting from oxidized LDL (ox-LDL) in a HUVEC model.[176] Allicin was responsible for a "browning" of white adipocytes in an *in vitro* model by enhancing the expression of brown adipocyte-specific genes, a finding with implications for metabolic disease, supported by a human clinical trial in which garlic improved multiple components of the metabolic syndrome.[177,178] A review of randomized and double-blinded studies show a consistent hypotensive effect, with the potential for cardiovascular risk reduction.[179]

Safety Summary: No known warnings, precautions or contraindications at the dose recommended.[85] Caution advised if risk of bleeding disorder present.[180] No adverse effects expected during pregnancy and breastfeeding.[85,181]

Grape Seed (*Vitis vinifera*)

Biological Actions:

Antimicrobial, anti-inflammatory, antioxidant, antibiofilm.

Scientific Evidence:

Grape seeds are a rich source of bioactive polyphenols, including proanthocyanidins, anthocyanins, flavonoids (flavonols and flavan-3-ols), stilbenes (resveratrol) and phenolic acids. Many of these polyphenols are known to have antioxidant effects, both upregulating antioxidant enzymes and directly neutralizing reactive oxygen species (ROS).[189] *In vitro*, grape seed extract (GSE) has been shown to decrease ROS intracellularly as well as within the mitochondria, and increase the expression of several antioxidant genes (GSR, SOD1, SOD2, and GPX2), as well as suppress the production of pro-inflammatory cytokines and restore the expression of tight junction proteins (ZO1, occludin, and claudin 1) following exposure to LPS.[182] In animal models, a poorly bioavailable grape polyphenol extract (with beta-carotene) was found not only to reduce the level of intestinal ROS in response to a high fat diet, but also to reduce several markers of inflammation (TNFα, IL-6, i-NOS), to promote the growth of beneficial bacteria (*Akkermansia muciniphila*), and to decrease the proportion of Firmicutes to Bacteroidetes, associated with a

[175] Zhang M, Pan H, Xu Y, et al. Allicin Decreases Lipopolysaccharide-Induced Oxidative Stress and Inflammation in Human Umbilical Vein Endothelial Cells through Suppression of Mitochondrial Dysfunction and Activation of Nrf2. Cell Physiol Biochem. 2017;41(6):2255-2267.
[176] Chen X, Pang S, Lin J, et al. Allicin prevents oxidized low-density lipoprotein-induced endothelial cell injury by inhibiting apoptosis and oxidative stress pathway. BMC Complement Altern Med. 2016 May 20;16:133.
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[182] Nallathambi R, Poulev A, Zuk JB, et al. Proanthocyanidin-Rich Grape Seed Extract Reduces Inflammation and Oxidative Stress and Restores Tight Junction Barrier Function in Caco-2 Colon Cells. Nutrients. 2020 Jun 1;12(6):1623.

metabolic benefit.[183,184] A systematic review of the effect of grape and red wine polyphenols on the microbiota concluded that both grape polyphenols modify the microbiome and that the ingested polyphenols are modulated by the gut microbiome, suggesting that grape seeds' physiological effects may be intertwined with the microbiome's composition.[185]

GSEs have demonstrated antimicrobial activity against several respiratory pathogens including *Moraxella catarrhalis*, *Staphylococcus aureus*, *Enterococcus faecalis, Streptococcus* sp. Group F*, Streptococcus pneumoniae and Pseudomonas aeruginosa*.[186] GSE has also demonstrated antibacterial activity against MRSA strains when assayed through *in vitro* experiments. While the underlying mechanism has not been fully elucidated, grape seed appears to reduce microbial growth by disrupting or breaking down cell wall surfaces.[187] An *in vitro* study found that at a low concentration, GSE disrupted the membranes of *Borrelia burgdorferi sensu lato,* while at a higher concentration, bacteria and cysts completely disappeared, leaving only fragments.[188] An *in vitro* study of Biocidin® LSF also documented killing of *Borrelia burgdorferi* spirochetes, with significant reductions 2 hours post-treatment. Inhibited growth of round bodies and biofilms over a 3-week period was also observed.[189] Other *in vitro* studies suggest that GSE also has the potential to protect against dental caries, demonstrating an ability to inhibit the growth of *Streptococcus mutans* as well as its formation of biofilms, and to reduce periodontal inflammation.[190,191,192]

The anti-inflammatory and antioxidant effects of GSE also have been associated with cardiometabolic changes. In a systematic review and meta-analysis of 50 randomized controlled trials, GSE was associated with improvements in glycemic and lipid profiles as well as markers of inflammation.[193] A similar analysis of 16 randomized trials found a hypotensive effect of GSE, particularly among young or obese subjects, or those with metabolic disorders.[194]

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[183] Kuhn P, Kalariya HM, Poulev A, et al. Grape polyphenols reduce gut-localized reactive oxygen species associated with the development of metabolic syndrome in mice. PLoS One. 2018 Oct 11;13(10):e0198716.
[184] Roopchand DE, Carmody RN, Kuhn P, et al. Dietary Polyphenols Promote Growth of the Gut Bacterium Akkermansia muciniphila and Attenuate High-Fat Diet-Induced Metabolic Syndrome. Diabetes. 2015 Aug;64(8):2847-58.
[185] Nash V, Ranadheera CS, Georgousopoulou EN, et al. The effects of grape and red wine polyphenols on gut microbiota - A systematic review. Food Res Int. 2018 Nov;113:277-287.
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[190] Zhao W, Xie Q, Bedran-Russo AK, et al. The preventive effect of grape seed extract on artificial enamel caries progression in a microbial biofilm-induced caries model. J Dent. 2014 Aug;42(8):1010-8.
[191] Bogdan C, Pop A, Iurian SM, et al. Research Advances in the Use of Bioactive Compounds from Vitis vinifera By-Products in Oral Care. Antioxidants (Basel). 2020 Jun 8;9(6):502.
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[193] Asbaghi O, Nazarian B, Reiner Ž, et al. The effects of grape seed extract on glycemic control, serum lipoproteins, inflammation, and body weight: A systematic review and meta-analysis of randomized controlled trials. Phytother Res. 2020 Feb;34(2):239-253.
[194] Zhang H, Liu S, Li L, et al. The impact of grape seed extract treatment on blood pressure changes: A meta-analysis of 16 randomized controlled trials. Medicine (Baltimore). 2016 Aug;95(33):e4247.

Safety Summary: No known warnings, precautions or contraindications at the dose recommended.[13] Exercise caution during pregnancy and breastfeeding as safety has not been established during these times.[13]

Black Walnut (*Juglans nigra*)

Biological Actions:

Anthelmintic, antimicrobial, antioxidant, antibiofilm.

Scientific Evidence:

The main active constituents of black walnut include naphthoquinones (juglone and plumbagin), tannins (ellagic acid and tannic acid) and flavanoids.[11,85,195] Black walnut hull and kernels have recently been shown to contain phenolics, including the antioxidants penta-O-galloyl-β-d-glucose, epicatechin gallate, quercetin, (-)-epicatechin, rutin, quercetin 3-β-d-glucoside, gallic acid, (+)-catechin, ferulic acid, and syringic acid, many of which have established physiological effects.[196,197,198]

Several compounds in black walnut have been found to have antimicrobial activity, including quercetin-3-O-glucoside (aka isoquercitin, eriodictyol-7-O-glucoside, quercetin, azelaic acid, and glansreginin A, demonstrating antibacterial activity against the Gram-positive bacterium (*S. aureus*).[199] Individually many of these compounds have shown broad antimicrobial activity, e.g. quercetin-3-O-glucoside has demonstrated antifungal activity, disrupting the membrane of Candida albicans; eriodictyol-7-O-glucoside has an antibacterial effect on the Gram-positive bacteria *Micrococcus luteus,* and *Staphylococus aureus*.[200,201] Juglone has demonstrated anti-bacterial and anti-parasitic activity against a variety of organisms *in vitro*, and inhibited both the formation of new biofilms as well as biofilm formation in *Candida albicans*.[202,203,204,205] The naphthoquinone plumbagin may also have anti-parasitic activity, inhibiting growth of *Leishmania*

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[195] Amarowicz R, Dykes GA, Pegg RB. Antibacterial activity of tannin constituents from Phaseolus vulgaris, Fagoypyrum esculentum, Corylus avellana and Juglans nigra. Fitoterapia. 2008 Apr;79(3):217-9.
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[199] Ho KV, Lei Z, Sumner LW, et al. Identifying Antibacterial Compounds in Black Walnuts (Juglans nigra) Using a Metabolomics Approach. Metabolites. 2018 Sep 29;8(4):58.
[200] Yun J, Lee H, Ko HJ, et al. Fungicidal effect of isoquercitrin via inducing membrane disturbance. Biochim Biophys Acta. 2015 Feb;1848(2):695-701.
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[202] Jha BK, Jung HJ, Seo I, et al. Juglone induces cell death of Acanthamoeba through increased production of reactive oxygen species. Exp Parasitol. 2015 Dec;159:100-6.
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[204] Wianowska D, Garbaczewska S, Cieniecka-Roslonkiewicz A, et al. Comparison of antifungal activity of extracts from different Juglans regia cultivars and juglone. Microb Pathog. 2016 Nov;100:263-267.
[205] Gumus B, Acar T, Atabey T, et al. The battle against biofilm infections: juglone loaded nanoparticles as an anticandidal agent. J Biotechnol. 2020 Jun 10;316:17-26.

donovani, and the nematodes *Caenorhabditis elegans*, *Haemonchus contortus* and *Ascaris suum*.[206,207,208]

Safety Summary:

No known warnings, precautions or contraindications at the dose recommended.[85]

Contraindicated during pregnancy and breastfeeding in therapeutic doses.[209]

Raspberry (*Rubus idaeus*)

Biological Actions:

Anti-inflammatory, antimicrobial, antioxidant, antibiofilm.

Scientific Evidence:

Raspberry is rich in anthocyanins (mainly cyanidin-3-sophoroside) and phenolic compounds (primarily ellagitannins and ellagic acid). Raspberry also contains quercetin and kaempferol-based flavanols.[210,211,212] Research shows that antioxidant properties of raspberry are attributed to its polyphenolic compounds, specifically ellagitannins, which are highly effective free radical scavengers. Results of an *in vitro* study indicate that raspberry's phenolics are able to protect DNA and decrease lipid peroxidation of lymphocytes in a concentration dependent manner.[218]

The active ellagitannin constituents (sanguiin H-6 and lambertianin C) have also demonstrated anti-inflammatory properties. Based on *in vitro* research, they inhibit the increase of NF-κB driven nuclear transcription and resultant TNFα production in a dose dependent manner.[213] In a small randomized crossover trial in humans, raspberry consumption was associated with an increase in flow-mediated dilation (a marker of endothelial function) up to 24 hours after ingestion, an effect attributed to ellagitannin.[214] An animal model highlighted the protective effect ellagic acid has against oxidant-induced endothelial dysfunction, which appeared to be mediated via Nrf2 activation, suggesting broad antioxidant effects.[215] A second model found a protective effect of

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[206] Fetterer RH, Fleming MW. Effects of plumbagin on development of the parasitic nematodes Haemonchus contortus and Ascaris suum. Comp Biochem Physiol C Comp Pharmacol Toxicol. 1991;100(3):539-42.
[207] Chaweeborisuit P, Suriyonplengsaeng C, Suphamungmee W, et al. Nematicidal effect of plumbagin on Caenorhabditis elegans: a model for testing a nematicidal drug. Z Naturforsch C J Biosci. 2016;71(5-6):121-31.
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[215] Ding Y, Zhang B, Zhou K, et al. Dietary ellagic acid improves oxidant-induced endothelial dysfunction and atherosclerosis: role of Nrf2 activation. Int J Cardiol. 2014 Aug 20;175(3):508-14.

ellagic acid against the development of diet-induced metabolic disease, with a corresponding normalization of Nrf2 and NF-κB levels.[216] In a 4 week randomized cross-over study, participants with obesity and diabetes had a significant lowering of the inflammatory markers IL-6 and high-sensitivity TNFα (but not C-reactive protein), as well as a blunting in post-prandial glucose following raspberry consumption versus control. The authors attributed this benefit to the effect of raspberry anthocyanins and ellagitannins on glucose transporters, as well as an inhibition of α-glucosidase activity, and an increase in insulin sensitivity.[217] The effects on insulin sensitivity were also supported by a single-blinded randomized controlled trial among participants at risk for diabetes; a post-prandial blunting effect on both glucose and insulin was observed following raspberry consumption vs. control.[218]

Phenolic compounds also possess antimicrobial properties and have been shown to inhibit the growth of both Gram-positive and Gram-negative pathogenic bacterial strains including *Staphylococcus aureus* and *Salmonella enterica* sp., as well as *Staphylococcus epidermidis,Helicobacter pylori, Bacillus cereus, Campylobacter jejuni* and *Candida albicans*.[23,25,219,220] The mechanism by which phenolic compounds affect the growth of different bacterial species include destabilization of cytoplasmic membrane, permeabilization of plasma membrane and inhibition of extracellular microbial enzymes. They also have direct actions on microbial metabolism by depriving the cells of the substrates necessary for growth.[26] Adherence of bacteria to epithelial surfaces is a prerequisite for colonization of many pathogens, therefore the antimicrobial activity of raspberry may be related in part to anti-adherence activity as suggested by Puupponen et al.[23] Growth of the probiotic strain *Lactobacillus rhamnosus* does not appear to be inhibited by the phenolic properties of raspberry.[25,227]

Safety Summary:

No known warnings, precautions or contraindications at the dose recommended. Take away from alkaloid-containing medications, metal ion supplements and vitamin B1 (thiamine).[85] No adverse effects expected during pregnancy and breastfeeding.[85]

Fumitory (*Fumaria officinalis*)

Biological Actions:

Antimicrobial, antioxidant.

Scientific Evidence:

The active constituents of fumitory include alkaloids, flavonoids, and organic acids.[11] The biological activities of this herb are mainly associated with the isoquinoline alkaloids, in particular

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[216] Panchal SK, Ward L, Brown L. Ellagic acid attenuates high-carbohydrate, high-fat diet-induced metabolic syndrome in rats. Eur J Nutr. 2013 Mar;52(2):559-68.
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protopine.[221,222] The antioxidant capacity of fumitory, however, is thought to be due to the synergistic effect of its constituents.[223] Both antioxidant activity and an inhibition of alpha-amylase were documented in an animal model, suggesting a possible role in glucose regulation.[224] *In vivo* and *ex vivo* animal models using extracts of fumitory also found reductions in the pro-inflammatory mediators TNFα and IL-6, and in a separate model, an increase in the anti-inflammatory factor IL-10, as well as protection of mitochondrial and hepatic function after exposure to permethrin.[225,226]

While the scientific evaluation of this herb is somewhat limited, an *in vitro* study assessing a methanol extract of fumitory demonstrated significant antimicrobial activity against the following microorganisms; *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Cladosporium herbarum*.[231] Reviews of the alkaloids found in Fumaria species suggests possible anti-viral, anti-biofilm, hepatoprotective, antifungal, and gastroprotective effects, but more research is needed to substantiate these findings.[227,228]

Safety Summary:

No known warnings, precautions or contraindications at the dose recommended.[229] Exercise caution during pregnancy and breastfeeding as safety has not been established during these times.[230,231]

Gentian (*Gentiana lutea*)

Biological Actions:

Anti-inflammatory, antimicrobial, antioxidant, antibiofilm.

Scientific Evidence:

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[221] Hentschel C, Dressler S, Hahn EG. Fumaria officinalis (Echter Erdrauch)--klinische Anwendung [Fumaria officinalis (fumitory)--clinical applications]. Fortschr Med. 1995 Jul 10;113(19):291-2. German.
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[223] Sengul M, Yildiz H, Gungor N, et al. Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. Pak J Pharm Sci. 2009 Jan;22(1):102-6.
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[226] Aoiadni N, Ayadi H, Jdidi H, et al. Flavonoid-rich fraction attenuates permethrin-induced toxicity by modulating ROS-mediated hepatic oxidative stress and mitochondrial dysfunction ex vivo and in vivo in rat. Environ Sci Pollut Res Int. 2021 Feb;28(8):9290-9312.
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[231] Assessment report on Fumaria officinalis L., herba (PDF) (Report). European Medicines Agency, Committee on Herbal Medicinal Products (HMPC). EMA/HMPC/576232/2010. Accessed 6-28-2021.

Gentian contains several secoiridoid bitter compounds including gentisin, gentiopicrin, amarogentin, gentianine, gentianadine, sweroside and swertiamarin. The medicinal constituents also include a group of xanthones (isovitexin and isogentisin) as well as phenolic acids and phytosterol flavonoids.[11,240,232] These active constituents give rise to the herb's potent antioxidant, anti-inflammatory and antibacterial properties.[240] Many *in vitro* and *in vivo* studies have documented the specific effects of Gentian's components individually as well as in combination; an *in vitro* study found that in adipocytes, gentianine (a metabolite of swertiamarin) upregulated the expression of PPAR-γ, GLUT-4 and adiponectin, an effect likely to improve glucose regulation.[233] Amarogentin, one of the most bitter naturally occurring substances, has been found to activate AMP-activated protein kinase (AMPK) and improve the homeostasis model assessment-insulin resistance (HOMA-IR) in animal models of diabetes.[234,235] Isovitexin, as well as an extract of gentian, reduced activity of several enzymes associated with inflammation-induced atherosclerosis, including vascular cell adhesion molecule-1 (VCAM-1), i-NOS, and vascular endothelial cadherin (VE-cadherin), with a reduction in lipid accumulation in the aorta of diabetic animals.[236]

In a small, controlled trial, gentian increased peripheral vascular resistance without increasing post-prandial blood pressure compared to control. This may allow for an increase in post-prandial intestinal blood flow, particularly during the intestinal phase of digestion.[237,238]

The antioxidant and cytoprotective action of gentian is at least partly due to the herb's ability to scavenge reactive oxygen species such as hydroxyl radicals thereby reducing free radical injury to cells.[241,239] Based on *in vitro* trials, the anti-inflammatory activity arises from gentian's ability to inhibit myeloperoxidase enzymes which are released during degranulation of neutrophils and monocytes. Myeloperoxidase up-regulation is known to contribute to the development of inflammatory and immune-mediated conditions.[241]

An *in vitro* analysis of gentian's antimicrobial properties found inhibition of the growth of both Gram-positive and Gram-negative organisms bacteria including *Listeria monocytogenes, Staphylococcus aureus*, *Salmonella typhimurium, Escherichia coli, Pseudomonas aeruginosa* and *Micrococcus luteus,* as well as *Candida albicans.* Although the widest spectrum of activity was attributed to gentiopicrin, a dominant role for any individual component was not observed, but rather a synergistic antimicrobial effect among gentian's constituents.[240]

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[232] Calliste CA, Trouillas P, Allais DP, et al. Free radical scavenging activities measured by electron spin resonance spectroscopy and B16 cell antiproliferative behaviors of seven plants. J Agric Food Chem. 2001 Jul;49(7):3321-7.
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[240] Savikin K, Menković N, Zdunić G, et al. Antimicrobial activity of Gentiana lutea L. extracts. Z Naturforsch C J Biosci. 2009 May-Jun;64(5-6):339-42.

Safety Summary:

No other known warnings, precautions or contraindications at the dose recommended.[12] No adverse effects expected during pregnancy and breastfeeding.[85]

Tea Tree oil (*Melaleuca alternifolia*)

Biological Actions:

Antifungal, antimicrobial, anti-parasitic, antibiofilm.

Scientific Evidence:

Tea tree oil (TTO) is composed of a complex mixture of compounds, mainly monoterpene and sesquiterpene hydrocarbons and their associated alcohols, such as α-pinene, sabinene, α-terpinene, limonene, p-cymene, 1,8-cineole, γ-terpinene, terpinolene, terpinen-4-ol (T4O), α-terpineol, aromadendrene, ledene, δ-cadinene, globulol and viridiforol.[241] The diverse active constituents give rise to tea tree's antimicrobial activity against a wide range of Gram-positive and Gram-negative bacteria as well as yeast and fungi.[256]

The main antibacterial constituents of TTO are terpinen-4-ol and γ-terpinene. It has been indicated that terpene compounds found in TTO act on the phospholipid layer of the microbial cell membrane, destroying its normal structure and function.[255] Transmission electron microscopy images reveal that TTO penetrates through the cell wall and cytoplasmic membrane of bacteria and fungi, causing damage to these structures, organelle damage, and ultimately death.[242] TTO has demonstrated consistent effective antimicrobial activity against MRSA, vancomycin-resistant *Enterococcus*, multi-resistant *Pseudomonas aeruginosa*, extended spectrum-beta-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae*.[243] The antimicrobial effect of TTO against *Pseudomonas aeruginosa* was found to be superior to commercially used antibacterial agents (specifically 0.1% chlorhexidine and 70% ethanol solutions).[258] In addition to being bactericidal and bacteriostatic against many oral pathogens, it also reduced gingival inflammation, comparable to chlorhexidine.[244] TTO has also been shown to decolonize and eradicate biofilms from *Staphylococcus aureus* (both coagulase-negative and coagulase-positive strains).[251] Numerous *in vitro* studies have demonstrated the potent antifungal activity of TTO against *Candida albicans* in both planktonic and biofilm culture. TTO has also demonstrated antifungal activity against *Saccharomyces uvarum* and *Trichophyton rubrum*.[250,252,254]

TTO also has several mechanisms for anti-parasitic activity; TTO can disrupt the protozoal plasma membrane, 1,8-cineole competitively blocks acetylcholinesterases (AChE), leading to a spastic paralysis in nematodes, while T4O inhibits histamine-induced edema and skin

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[241] Lam NS, Long X, Su XZ, et al. Melaleuca alternifolia (tea tree) oil and its monoterpene constituents in treating protozoan and helminthic infections. Biomed Pharmacother. 2020 Oct;130:110624.
[242] Li WR, Li HL, Shi QS, et al. The dynamics and mechanism of the antimicrobial activity of tea tree oil against bacteria and fungi. Appl Microbiol Biotechnol. 2016 Oct;100(20):8865-75.
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[244] Casarin M, Pazinatto J, Santos RCV, et al. Melaleuca alternifolia and its application against dental plaque and periodontal diseases: A systematic review. Phytother Res. 2018 Feb;32(2):230-242.

inflammation, preventing many parasites from establishing a suitable environment.[245,246,247] TTO, particularly T4O, also has miticidal effects, and has been shown to both lower mite counts and relieve Demodex-related symptoms.[248] Finally, TTO may also have an anti-viral effect. In a small randomized clinical trial, children with molluscum contagiosum received topical iodine, topical TTO, or a combination of the two. The combined effect was substantially greater than either treatment alone; efficacy, defined as a greater than 90% reduction in the number of lesions after 30 days, was achieved in 84% of the combination group, vs. only 6% and 17% in the iodine and TTO only groups, respectively.[249] While the TTO may have enhanced the penetration of the iodine, other studies suggest it may also have intrinsic anti-viral properties.[250]

Safety Summary:

Considered safe and well tolerated at the dose recommended. TTO is generally regarded as non-toxic, and non-irritating, though a small number of individuals have an allergy to TTO oxidation products.[251] Exercise caution during pregnancy and breastfeeding as safety has not been established during these times.[252]

Galbanum oil (*Ferula galbaniflua*)

Biological Actions:

Anti-inflammatory, antimicrobial, antiseptic.

Scientific Evidence:

Galbanum is composed of mainly monoterpene, sesquiterpene hydrocarbons, and their associated alcohols. It is the high concentrations of monoterpenes and sesquiterpenes that give rise to galbanum's anti-inflammatory, antimicrobial, and antiseptic properties, with monoterpene hydrocarbons comprising over 95% of Galbanum oil.[268,253,254] Often considered synonymous with *Ferula gummosa,* these species are distinct, with greater amounts of feselol and sterol compounds found in *Ferula galbaniflua*. Feselol has been shown to inhibit *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* in vitro.[255]

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[245] Mills C, Cleary BJ, Gilmer JF, Walsh JJ. Inhibition of acetylcholinesterase by Tea Tree oil. J Pharm Pharmacol. 2004 Mar;56(3):375-9.
[246] Wolstenholme AJ. Ion channels and receptor as targets for the control of parasitic nematodes. Int J Parasitol Drugs Drug Resist. 2011 Oct 14;1(1):2-13.
[247] de Oliveira MP, Lima MC, Calheiros AS, et al. Leishmania (Viannia) braziliensis: human mast cell line activation induced by logarithmic and stationary promastigote derived-lysates. Exp Parasitol. 2005 Feb;109(2):72-9.
[248] Lam NSK, Long XX, Li X, et al. Comparison of the efficacy of tea tree (Melaleuca alternifolia) oil with other current pharmacological management in human demodicosis: A Systematic Review. Parasitology. 2020 Dec;147(14):1587-1613.
[249] Markum E, Baillie J. Combination of essential oil of Melaleuca alternifolia and iodine in the treatment of molluscum contagiosum in children. J Drugs Dermatol. 2012 Mar;11(3):349-54.
[250] Li X, Duan S, Chu C, et al. Melaleuca alternifolia concentrate inhibits in vitro entry of influenza virus into host cells. Molecules. 2013 Aug 9;18(8):9550-66.
[251] Hammer KA, Carson CF, et al. A review of the toxicity of Melaleuca alternifolia (tea tree) oil. Food Chem Toxicol. 2006 May;44(5):616-25.
[252] Natural Standard. Professional Monograph: Tea tree oil (Melaleuca alternifolia). http://www.naturalstandard.net. Accessed December 24th, 2013.
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[254] Kanani MR, Rahiminejad MR, Sonboli A, et al. Chemotaxonomic significance of the essential oils of 18 Ferula species (Apiaceae) from Iran. Chem Biodivers. 2011 Mar;8(3):503-17.
[255] Amin A, Hanif M, Abbas K, et al. Studies on effects of umbelliferon derivatives against periodontal bacteria; antibiofilm, inhibition of quorum sensing and molecular docking analysis. Microb Pathog. 2020 Jul;144:104184.

Terpenes have been shown to be active against bacteria, fungi, viruses, and protozoa. The mechanism by which terpenes exert their antimicrobial properties involves disruption of the lipophilic compounds of cellular membranes of pathogens.[256] Galbanum oil has demonstrated a cytotoxic effect *in vitro* against the protozoa *Leishmania amazonensis*, with a higher selectivity index than 15 other essential oils tested. This may at least in part be due to the presence of the monoterpene β-pinene.[257]

To date, few scientific studies have been conducted with galbanum oil. Traditionally the herb has been used in the treatment of inflammatory and skin disorders, in wound healing and for ailments of the respiratory, digestive, and nervous systems.[271]

Safety Summary:

Galbanum oil is generally regarded as non-toxic, non-irritating and non-sensitizing.[268] Exercise caution during pregnancy and breastfeeding as safety has not been established during these times.[258]

Lavender oil (*Lavandula officinalis*)

Biological Actions:

Antifungal, anti-inflammatory, antimicrobial, antibiofilm.

Scientific Evidence:

Lavender oil contains a complex mixture of aromatic compounds, specifically terpenes and sesquiterpenes which include linalyl acetate, linalool, caryophyllene, terpinen-4-ol, 2-myrcene, trans-ocimene, borneol, 1,8-cineole, camphor and limonene.[278,259,260]

This essential oil has been found to be active against many species of bacteria and fungi. Based on *in vitro* studies, lavender oil has demonstrated antibacterial activity against both methicillin-sensitive and methicillin-resistant strains of *Staphylococcus aureus*.[278,279,261] Lavender oil has demonstrated both fungistatic and fungicidal activity against *Candida albicans.* Research also shows lavender is effective against both vaginal and oropharyngeal strains of *Candida albicans.* In a study by D'Auria et al, lavender oil inhibited both germ tube formation and hyphal elongation of *Candida albicans.*[277]

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[256] Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999 Oct;12(4):564-82.
[257] Andrade MA, Azevedo CD, Motta FN, et al. Essential oils: in vitro activity against Leishmania amazonensis, cytotoxicity and chemical composition. BMC Complement Altern Med. 2016 Nov 8;16(1):444.
[258] Natural Medicines Comprehensive Database. Galbanum Monograph. http://naturaldatabase.therapeuticresearch.com. Accessed December 25th, 2013.
[259] Evandri MG, Battinelli L, Daniele C, et al. The antimutagenic activity of Lavandula angustifolia (lavender) essential oil in the bacterial reverse mutation assay. Food Chem Toxicol. 2005 Sep;43(9):1381-7.
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[261] Nelson RR. In-vitro activities of five plant essential oils against methicillin-resistant Staphylococcus aureus and vancomycin-resistant Enterococcus faecium. J Antimicrob Chemother. 1997 Aug;40(2):305-6.

Based on *in vitro* experiments, lavender helps protect against LPS-induced inflammation from Gram-negative bacteria. Exposure to LPS in tissues induces an inflammatory reaction which triggers the release of proinflammatory cytokines and subsequent free radical pathology. Research by Huang et al verified lavender oil was able to inhibit LPS-dependent superoxide anion generation, NF-KB activation and IL-1β production.[276]

Lavender oil has also been found to significantly increase levels of TGF-β and type I collagen, increasing wound contraction and tissue remodeling, thereby accelerating the rate of wound healing.[262] It has also been reported to upregulate epidermal growth factor (EGF) synthesis, potentially contributing to more rapid healing.[263] The modulation of GABAA receptors by linalool, the primary mechanism for the anxiolytic effect of lavender, may occur with both inhaled and ingested lavender.[264,265,266,267]

Safety Summary:
Lavender oil is generally regarded as non-toxic, non-irritant, and non-sensitizing.[268] No adverse effects expected during pregnancy and breastfeeding at the dose recommended.[268]

Oregano oil (*Origanum vulgare*)

Biological Actions:
Antibacterial, antifungal, anti-inflammatory, antioxidant, antibiofilm.

Scientific Evidence:
Active constituents of oregano oil include monoterpenes and sesquiterpene hydrocarbons, as well as phenolic compounds; the main terpenes are thymol, carvacrol, p-cymene, γ-terpinene, and linalool.[269]

Based on *in vitro* research, oregano oil showed inhibitory effect against multiple organisms, against both Gram-positive and Gram-negative bacteria, including *Listeria monocytogenes, Escherichia coli, Salmonella enteritidis, Pseudomonas aeruginosa, Staphylococcus aureus* and erythromycin-resistant Group A *Streptococcus pyogenes*.[291] Both carvacrol and oregano oil were

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[262] Samuelson R, Lobl M, Higgins S, et al. The Effects of Lavender Essential Oil on Wound Healing: A Review of the Current Evidence. J Altern Complement Med. 2020 Aug;26(8):680-690.
[263] Koca Kutlu A, Ceçen D, Gürgen SG, et al. A Comparison Study of Growth Factor Expression following Treatment with Transcutaneous Electrical Nerve Stimulation, Saline Solution, Povidone-Iodine, and Lavender Oil in Wounds Healing. Evid Based Complement Alternat Med. 2013;2013:361832.
[264] Milanos S, Elsharif SA, Janzen D, et al. Metabolic Products of Linalool and Modulation of GABAA Receptors. Front Chem. 2017 Jun 21;5:46.
[265] Kasper S, Gastpar M, Müller WE, et al. Silexan, an orally administered Lavandula oil preparation, is effective in the treatment of 'subsyndromal' anxiety disorder: a randomized, double-blind, placebo controlled trial. Int Clin Psychopharmacol. 2010 Sep;25(5):277-87.
[266] Kasper S. An orally administered lavandula oil preparation (Silexan) for anxiety disorder and related conditions: an evidence based review. Int J Psychiatry Clin Pract. 2013 Nov;17 Suppl 1:15-22.
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[268] Brendler T, Gruenwald J, Jaenicke C. Comm. E Monograph: Lavandulae flos In: Heilpflanzen - Herbal Remedies. In: Publishers MS, ed; 2003. Accessed December 25th, 2013.
[269] Lombrea A, Antal D, Ardelean F, et al. A Recent Insight Regarding the Phytochemistry and Bioactivity of Origanum vulgare L. Essential Oil. Int J Mol Sci. 2020 Dec 17;21(24):9653.

also found to inhibit the growth of *Borrelia burgdorferi in vitro,* and dissolve its biofilms to a greater degree than 33 other essential oils tested.[270] In an animal model, oregano oil was found to have significant antibacterial activity against 11 multi-drug resistant strains, and effectively eradicated the biofilms for each pathogen tested.[271] *In vitro* testing found oregano oil to inhibit all 59 strains of bacteria isolated from subjects with cystic fibrosis, many of which were also multi-drug resistant.[272] This broad antibacterial activity suggests the possibility that oregano oil has multiple cellular targets, though it is clear that both thymol and carvacrol target bacterial membrane proteins, increasing the permeability of the cell membrane.[290,291]

Oregano oil has also demonstrated antifungal activities against *Candida* species.[273] In the study by Pozzatti et al, oregano inhibited the growth and hyphenation of both *Candida albicans* and *Candida dubliniensis*.[274] One mechanism of its antifungal activity is associated with the lipophilicity of oregano oil and consequent interaction with the microbial cell membrane. The lipophilic nature of the oil results in changes and losses of enzymatic and structural components of fungal cells.[296] Additionally, thymol and carvacrol overwhelm *Candida* antioxidant defense systems with an oxidative radical cascade, inhibit ergosterol synthesis, and target efflux pump activity.[275,276]

Animal and *in vitro* models suggest an antioxidant and anti-inflammatory component to oregano oil as well. In one animal model, oregano oil protected intestinal tissue from oxidative damage, reduced expression of TNFα and IL-6, and increased occludin expression, suggesting it provided protection from oxidative stress and inflammation, and functional loss of the intestinal barrier.[277] *In vitro* and *in vivo* models also cite a protective effect of carvacrol against oxidative stress and inflammation, in part mediated via an induction of Nrf2 activity, as well as inhibition of the NLRP3 inflammasome.[278,279,280] A small clinical trial found a reduction in several pro-inflammatory

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[270] Feng J, Zhang S, Shi W, et al. Selective Essential Oils from Spice or Culinary Herbs Have High Activity against Stationary Phase and Biofilm Borrelia burgdorferi. Front Med (Lausanne). 2017 Oct 11;4:169.
[271] Lu M, Dai T, Murray CK, et al. Bactericidal Property of Oregano Oil Against Multidrug-Resistant Clinical Isolates. Front Microbiol. 2018 Oct 5;9:2329.
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[274] Pozzatti P, Loreto ES, Nunes Mario DA, et al. Activities of essential oils in the inhibition of Candida albicans and Candida dubliniensis germ tube formation. Journal de Mycologie Médical 2010;20(3):185-189.
[275] Bae YS, Rhee MS. Short-Term Antifungal Treatments of Caprylic Acid with Carvacrol or Thymol Induce Synergistic 6-Log Reduction of Pathogenic Candida albicans by Cell Membrane Disruption and Efflux Pump Inhibition. Cell Physiol Biochem. 2019;53(2):285-300.
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[279] Naeem K, Tariq Al Kury L, Nasar F, et al. Natural Dietary Supplement, Carvacrol, Alleviates LPS-Induced Oxidative Stress, Neurodegeneration, and Depressive-Like Behaviors via the Nrf2/HO-1 Pathway. J Inflamm Res. 2021 Apr 8;14:1313-1329.
[280] Arruri VK, Gundu C, Kalvala AK, et al. Carvacrol abates NLRP3 inflammasome activation by augmenting Keap1/Nrf-2/p62 directed autophagy and mitochondrial quality control in neuropathic pain. Nutr Neurosci. 2021 Feb 28:1-16.

cytokines, comparing carvacrol to placebo, among veterans exposed to mustard gas decades before.[281]

Safety Summary:

Generally considered safe and well tolerated at the dose recommended. Active phenolic compounds such as thymol and carvacrol in oregano oil may, in some sensitive individuals, cause skin and mucus membrane irritation.[268] Exercise caution during pregnancy and breastfeeding as safety has not been established during these times.[282]

[281] Khazdair MR, Boskabady MH. The effect of carvacrol on inflammatory mediators and respiratory symptoms in veterans exposed to sulfur mustard, a randomized, placebo-controlled trial. Respir Med. 2019 Apr;150:21-29.
[282] Natural Medicines Comprehensive Database. Oregano Monograph. http://naturaldatabase.therapeuticresearch.com. Accessed December 25th, 2013.